

SEARCH REQUEST FORM

Jan
Examiner # (Mandatory): _____ Requester's Full Name: Howard Owens
Art Unit 1623 Location (Bldg/Room#): CM1/8D12 Phone (circle 305 ~~306~~ 308) 4538
Serial Number: 09/234,532 Results Format Preferred (circle): PAPER DISK E-MAIL
Title of Invention _____
Inventors (please provide full names): _____

Earliest Priority Date: _____
Keywords (include any known synonyms registry numbers, explanation of initialisms):

Search Topic:

Please write detailed statement of the search topic, and the concept of the invention. Describe as specifically as possible the subject matter to be searched. Define any terms that may have a special meaning. Give examples of relevant citations, authors, etc., if known. You may include a copy of the abstract and the broadcast or most relevant claim(s).

Please search claims 1-20.

STAFF USE ONLY

Searcher: _____	Type of Search	Vendors (include cost where applicable)
Searcher Phone #: <u>4495</u>	_____ N.A. Sequence	<u>✓</u> STN
Searcher Location: _____	_____ A.A. Sequence	_____ Questel/Orbit
Date Picked Up: <u>7/25</u>	_____ Structure (#)	_____ Lexis/Nexis
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	_____ Other	_____ Westlaw
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1 of 1 DOCUMENT

5,348,979

Sep. 20, 1994

Method of promoting nitrogen retention in humans

LIT-REEX: NOTICE OF LITIGATION

Met-Rx Substrate Technology, Inc. v. Metabolic Technologies, Inc., et al,
Filed May 3, 1999, D.C. C.D. California, Doc. No. CV99-4766 ABC (VAPx)

INVENTOR: Nissen, Steven L., Ames, Iowa
Flakoll, Paul J., Old Hickory, Tennessee
Abumrad, Naji N., Old Field, New York

ASSIGNEE-AT-ISSUE: Iowa State University Research Foundation Inc., Ames, Iowa
(02) , Vanderbilt University, Nashville, Tennessee (02)

ASSIGNEE-AFTER-ISSUE: Date Transaction Recorded: Feb. 22, 1999

LICENSE (SEE DOCUMENT FOR DETAILS).

MET-RX SUBSTRATE TECHNOLOGY, INC. 361 HOSPITAL ROAD, SUITE 413 NEWPORT BEACH,
CALIFORNIA 32663

Reel & Frame Number: 009773/0790

Date Transaction Recorded: Feb. 22, 1999

SECURITY INTEREST (SEE DOCUMENT FOR DETAILS).

LASALLE NATIONAL BANK 135 SOUTH LASALLE STREET CHICAGO, ILLINOIS 60603

Reel & Frame Number: 009773/0814

APPL-N0: 996,187

FILED: Dec. 23, 1992

INT-CL: [5] A61K 31#19; A61K 31#335; A23L 1#03

US-CL: 514#557; 426#2; 426#531; 514#449;

CL: 514;426;

SEARCH-FLD: 514#557, 449; 426#2, 531

REF-CITED:

U.S. PATENT DOCUMENTS

4,100,161	7/1978	*	Walser	424#274
4,100,293	7/1978	*	Walser	424#274
4,677,121	6/1987	*	Walser et al.	514#561
4,760,090	7/1988	*	Nissen	514#561
4,764,531	8/1988	*	Nissen	514#557
4,992,470	2/1991	*	Nissen	514#578
5,028,440	7/1991	*	Nissen	514#557
5,087,472	2/1992	*	Nissen	514#557

OTHER PUBLICATIONS

Walser et al., J. Clin. Inv. (1973) 52:678-690.
Saiper and Walser, Metabolism (1977) 26:301-308.
Chawla et al., J. Nutri. (1975) 105:798-803.
Boebel and Baker, J. Nutr. (1982) 112:1929-1939.
Tanaka et al., Biochim. Biosphys. Acta. 152:638-641 (1966).
Landass, Clin. Chim. Acta. 64:143-154 (1975).

PRIM-EXMR: Henley, III, Raymond J.

LEGAL-REP: Tilton, Fallon, Lungmus & Chestnut

ABST:

Nitrogen retention in human subjects is promoted by administering beta - hydroxy- beta -methylbutyric acid (HMB). The amount of HMB administered is effective to conserve protein as determined by reduction in urinary nitrogen. The method can be used with patients having a negative nitrogen balance due to disease conditions, and also with normal elderly persons who are subject to protein loss. The HMB may be administered orally or by intravenous infusion.

NO-OF-CLAIMS: 14

EXMPL-CLAIM: 1

NO-OF-FIGURES: 0

NO-DRWNG-PP: 0

SUM:

FIELD OF INVENTION

This invention relates to the promotion of nitrogen retention in humans, and more particularly to the administration of therapeutic agents for this purpose.
BACKGROUND OF INVENTION

Tissue proteins forms the basis for organ structure and function. Excessive losses of tissue protein can compromise organ function and eventually will result in death. Any stressful situation such as trauma and chronic debilitating diseases results in tissue losses that if sustained can compromise organ function. In most cases nutrition alone cannot prevent this tissue loss because of excessive breakdown of tissue proteins. Thus alternatives to nutrition must be used to abate or slow the protein wasting or excessive loss of body nitrogen.

Nitrogen balance is the difference between the nitrogen intake (as protein or amino acids) in an individual and the total nitrogen excretion. When the nitrogen intake equals the nitrogen excretion, the subject is in nitrogen equilibrium. If the nitrogen intake exceeds the nitrogen excretion, the nitrogen balance is positive, but if the nitrogen excretion is greater than the nitrogen intake, the nitrogen balance is negative. Nitrogen balance can be estimated by monitoring urinary nitrogen. Absolute nitrogen balance also requires fecal nitrogen measurement, but in most cases this does not change appreciably unless

the diet is substantially altered. Thus, the nitrogen content of urine can be approximately correlated with total nitrogen excretion. Monitoring nitrogen content of urine is especially important where the patient has or is expected to have a persistent negative nitrogen balance.

Promoting nitrogen retention has therapeutic importance where the patient has been subjected to trauma or stress conditions which can be expected to induce potential loss. Injury (surgical, traumatic, and burn) and sepsis result in accelerated protein breakdown, which is manifested by increased nitrogen loss. Catabolic conditions are also frequently associated with severe bodily diseases such as cancer, AIDS, etc. Loss of muscle protein may occur due to normal aging, and consequently, protein sparing therapy may be indicated for elderly patients who are otherwise normal.

Therapeutic agents and certain nutritional regimes are known which can promote nitrogen retention. However, therapeutic options to decrease body nitrogen losses (protein wasting) are limited. Injections of certain hormones may improve nitrogen retention. Growth hormone injections can in the short term at least decrease tissue protein losses: Horber et al., J. Clin. Invest. (1990) 86: 256. Steroids such as testosterone when injected can decrease nitrogen loss: Daham et al., Metabolism (1989) 38: 197. These compounds have to be injected and may have undesirable side effects, limiting usefulness in disease states.

A nutritional approach to protein sparing was investigated by Dr. MacKenzie Walser and associates. They experimented with keto analogs of essential amino acids as partial or complete substitutes for the corresponding amino acids, for example, as supplementation to protein-reduced diets in uremia. [See, for example, Walser et al., J. Clin. Inv. (1973) 52: 678-690.] Experiments by Walser and associates demonstrated a nitrogen sparing effect from mixtures of branched-chain keto acids: Saiper and Walser, Metabolism (1977) 26: 301-308. Patents have issued to Walser on the use of keto analogs of essential amino acids for promoting synthesis and suppression of urea formation in humans (U.S. Pat. Nos. 4,100,161 and 4,101,293).

The keto acid analog of L-leucine is alpha-ketoisocaproate (KIC) which is also sometime referred to as "keto-leucine". KIC does not have L and D forms as does leucine. It is known that there is an interconversion of circulating KIC and leucine. Published studies have demonstrated that KIC can be substituted in animal diets for leucine providing that larger molar amounts of KIC are used. Chawla et al. reported that weight loss by rats being fed a diet deficient in leucine could be prevented by adding KIC to the diet, but the efficiency of substitution was only 20 to 27%. [J. Nutr. (1975) 105: 798-803], and Boebel et al. reported that the efficiency of KIC was only about 56% with reference to leucine [Boebel and Baker, J. Nutr. (1982) 112: 1929-1939].

Dr. Steven L. Nissen of Iowa State University, Ames, Iowa, U.S.A. has carried out studies with domestic animals in which KIC is incorporated in the animal feeds. As described in his U.S. Pat. No. 4,760,090, it was found that ketoisocaproate (KIC) when fed to cattle and sheep can result in enhancement of growth and feed efficiency. In another use of KIC feeding, egg production of laying chickens was increased (U.S. Pat. No. 4,764,531). In later experiments carried by Dr. Nissen at Iowa State University, beta -hydroxy- beta -methylbutyric acid (HMB) was fed to domestic animals. The effects obtained with HMB feeding were different than with KIC.

Metabolically, KIC and HMB are not directly related. KIC is the only metabolic product of leucine, while HMB is a minor product in the metabolic

sequence of KIC. Leucine is either used for protein synthesis in the body or is converted directly to KIC. In the mitochondria, KIC is decarboxylated to isovalerylCoA and then further metabolized to ketone bodies. In certain disease conditions, such as isovaleric acidemia, an alternate oxidative pathway for KIC has been observed, which appears to produce beta -hydroxy- beta -methyl-butyrate (HMB). In atypical cases, such as a genetic absence of the dehydrogenase enzyme, there is evidence that HMB can accumulate in the urine: Tanaka, et al. Biochim. Biophys. Acta. 152: 638-641 (1968). Also, in acidosis conditions, HMB levels can be increased in urine: Landass, Clin. Chim. Acta. 64: 143-154 (1975).

The differing activities of HMB as fed to domestic animals provided the basis for several patents by Dr. Nissen. U.S. Pat. No. 4,992,470 discloses administration of HMB for enhancing the immune response of mammals, and/or as an ingredient in the raising of meat producing animals (viz. ruminants and poultry) to increase lean tissue development. (See also U.S. Pat. Nos. 5,087,472 and 5,028,440.)

SUMMARY OF INVENTION

This invention is based on the discovery that nitrogen retention in humans can be dramatically improved by the administration of small amounts of beta -hydroxy- beta -methylbutyric acid (HMB). In experiments which lead to the present invention, using normal human subjects nitrogen retention was increased by an average of 18%. This result was unexpected. Heretofore, as far as it is known, there have been no reports of experiments with either humans or animals in which KIC or HMB was administered and effects on nitrogen retention determined. The finding that the administration of one gram of HMB per subject per 24 hours appreciably reduced urinary nitrogen was especially significant since the subjects were well-nourished adults. Prior studies on other means of promoting nitrogen retention have been conducted mostly with subjects under stress conditions who had negative nitrogen balances. A substance that can increase nitrogen retention in humans who are not experiencing a negative nitrogen balance has manifest therapeutic potential.

In accordance with the present invention, HMB can be orally administered to human patients as a protein sparing therapy. At dosages effective for promoting nitrogen retention, HMB is not known to be toxic or to have any undesirable side effects. It can be safely administered to persons afflicted with trauma, stress or other catabolic condition, including people undergoing semi-starvation. HMB can also be used in conjunction with weight reduction programs where it is desired to minimize loss of tissue protein. Moreover, it is believed that HMB can be regularly incorporated in food supplements for the elderly, thereby tend to offset the protein losses which may occur in persons of advanced age. In general, the method of this invention can be used to improve nitrogen balance for human subjects whenever it is medically desirable to counter urinary nitrogen loss which cannot be overcome nutritionally.

DETDESC:

DETAILED DESCRIPTION

The base compound for practicing the present invention is beta -hydroxy- beta -methylbutyric acid (HMB). It can be used in its free acid form or as an edible salt, and edible derivatives of HMB which convert directly in the body to HMB can be used. The free acid compound is also called beta -hydroxy-isovaleryic acid. It has the following structure: [See Original Patent for Chemical Structure Diagram]

It is preferred to administer HMB as an edible salt, ester, or lactone. The calcium salt is especially convenient because it is less hygroscopic than the sodium or potassium salts. Esters of HMB such as particularly the methyl or ethyl esters are alternative forms. Such esters are rapidly converted in the body to free acid HMB. For administration as a lactone, the compound isovaleryl lactone can be used. This compound and similar lactones are rapidly converted in the body to free acid HMB.

The free acid form can be designated as "HMB-acid", and the salt forms, such as the calcium, sodium, potassium or magnesium salts, respectively, as "Ca-HMB", "Na-HMB", "K-HMB", and "Mg-HMB". Correspondingly, the esters can be designated "HMB-methyl ester", "HMB-ethyl ester", etc. The lactone can be designated "HMB-lactone". HMB has no stereo-isomers and accordingly does not exist in L or D forms.

HMB is not commercially available at this time. However, procedures are known for synthesizing this compound from commercially available starting materials. For example, HMB can be synthesized by oxidation of diacetone alcohol (4-hydroxy-4-methyl-2-pentanone). A suitable synthesis procedure is described by Coffman, et al., J. Am. Chem. Soc., 80: 2882-2887, at 2885 (1958). As there described, beta -hydroxy-isovaleryic acid (HMB) is prepared by an alkaline sodium hypochlorite oxidation of diacetone alcohol. The product is recovered in free acid form which can be converted to the desired salt. For example, HMB can be prepared as its calcium salt (Ca-HMB) by a similar procedure to that of Coffman, et al. in which the HMB acid obtained is neutralized with calcium hydroxide, and recovered by crystallization from an aqueous ethanol solution. For example, a 95% ethanol solution can be used with the Ca-HMB at about a 10% concentration.

Since Ca-HMB is a preferred form for administering HMB, the dosage amount of HMB can be expressed in terms of corresponding mole amount of Ca-HMB. The dosage range within which HMB can be usefully administered orally or intravenously for promoting nitrogen retention is within the range from 0.01 to 0.2 grams HMB (Ca-HMB basis) per kilogram of body weight per 24 hours. For adults, assuming body weights of from about 100 to 200 lbs., the dosage amount orally or intravenously of HMB (Ca-HMB basis) can range from 0.5 to 10 grams per patient per 24 hours. A presently preferred amount is from 2 to 6 grams HMB (Ca-HMB basis) per patient per 24 hours.

Ca-HMB and other forms of HMB as described above can be processed as fine powders which can be filled into capsules, or combined with tableting diluents, such as lactose, and compressed into tablets of predetermined dose amounts. No special mode of oral administration is needed. One preferred mode is to package the Ca-HMB in water-soluble capsules, such as gelatin capsules. Each capsule may contain as the predetermined amount of the Ca-HMB 0.5, 1, or 2 grams. Multiple doses per day are desirable, and therefore smaller dose sizes are believed preferable. However, if desired, larger doses in capsules or tablets can be prepared, such as 4 grams per capsule or tablet. A suitable regimen for oral administration to adults consists of one tablet or capsule one to four times per 24 hours. Corresponding amounts of HMB can be fed as an ingredient of solid or liquid dietary supplements, such as particularly supplements designed for use by the elderly. Alternatively, Ca-HMB can be dissolved in milk or fruit juice such as orange juice, resulting in a palatable drink.

HMB in a water-soluble non-toxic form can also be administered by intravenous

infusion. This method is particularly suitable for hospitalized patients that are on IV therapy. For example, Ca-HMB or Na-HMB can be dissolved in an intravenous solution being administered to the patient, viz. normal saline, glucose, etc. Ca-HMB or Na-HMB may also be added to nutritional IV solutions, which may include amino acids and lipids. The amounts to be administered intravenously can be similar levels to oral administration, but it is believed that a maximized protein retention effect should be obtainable at lower doses by infusion. Infusion also has the advantage that the HMB introduction can be metered and controlled more accurately. For example, beneficial results on nitrogen retention can be obtained by infusion of 0.5 to 10 grams per 24 hours, or preferably from 2 to 6 grams.

The experimental basis of the present invention and the results that can be obtained are further illustrated by the following experimental example.

EXPERIMENTAL EXAMPLE

Preparation of HMB

Ca-HMB was prepared by minor modification of the method of Coffman, et al. J. Am. Chem. Soc., 80: 2882-2887 (1958). More specifically, the crude HMB was first purified by distillation under vacuum, neutralized with Ca(OH)₂, and finally the calcium salt crystallization three times from 95% ethanol. The product was then air-dried and fine-ground. Each batch was given a lot number and the purity assessed by high performance liquid chromatography. A single peak was measured when HMB was chromatographed on a C18 column and eluted with 0.01M phosphate buffer, pH 7.0. Also nuclear magnetic resonance was performed. This indicated only two peaks which corresponded to the methyl hydrogens and the CH₂ hydrogens. The resulting purified Ca-HMB was used in a human subject study as follows.

Experimental Procedure

In a controlled double-blind human study, the effects of feeding HMB on loss of urinary nitrogen was tested. Effects on blood cholesterol and immune function were also observed. Male subjects (22 to 43 years of age) were used who had been screened for normalcy. They were well-nourished, healthy adults. Ca-HMB was administered in 250 mg capsules. The subjects were instructed to take the capsules in 4 equal doses daily (with meals and at bed time), giving a dose of one gram per subject per 24 hours. The subject ate all their meals under controlled conditions. Normal diets were used, but the amounts of the diets were controlled to maintain equal and substantially constant nitrogen intake. The subjects had blood drawn before the morning meal or before they took the morning HMB dose. Each subject was studied twice: once with a placebo and once with HMB. The subjects did not know which preparations they were given.

The baseline time period consisted of 5 days of controlled dietary treatment followed by a 14 day period of HMB treatment. Urine was quantitatively collected on the last 2 days of the baseline period and the last 4 days of the treatment period.

Urinary nitrogen was quantitated by the Kjeldahl method. The percent nitrogen times the urine volume resulted in grams of urine nitrogen excreted per day. Net change was calculated by subtracting the change during the HMB period from the placebo period. The results are summarized in the accompanying Table A.

TABLE A

	Placebo		HMB		% Change		Net %
	Baselin e	Treatmen t	Baselin e	Treatment	Plac	HMB	HMB Effect
Body Weight	167	167	176	179	0	1.4	1.4

Body fat (%)	12.4	12.0	12.5	11.0	- 3	- 12	- 9
Urine Nitrogen (g/d)	14.52	16.08	16.68	15.4	10.7	- 7.8	18.5
Blood Urea (mg/dl)	9.20	9.68	6.80	6.58	7.2	- 3.4	- 10.7

Results

As shown by the data of Table A, HMB decreased the average amount of urine nitrogen by 18% (statistically significant at the $p < 0.02$ level). All five subjects decreased urine nitrogen when fed HMB compared to the placebo period. Concomitant with this change was a decrease in blood urea nitrogen. This result suggests that HMB stimulated the retention of dietary protein in body, and presumably this resulted in increased tissue protein retention because dietary nitrogen intake was calculated to be at maintenance. HMB appears to be a potent agent for promoting nitrogen retention even in normal subjects.

CLAIMS: We claim:

[*1] 1. The method of protein sparing, comprising orally or intravenously administering to a human subject an effective amount of beta -hydroxy- beta -methylbutyric acid (HMB) for increasing the retention of nitrogen, said HMB being in an edible or intravenously-administrable form selected from (i) its free acid form, (ii) its sodium, potassium, or calcium salt, (iii) its methyl or ethyl ester, or (iv) its lactone, and continuing the said administration of HMB until the amount of nitrogen in the patient's urine has substantially decreased.

[*2] 2. The method of claim 1 in which said HMB is in the form of its calcium salt (Ca-HMB) or its sodium salt (Na-HMB).

[*3] 3. The method of claims 1 or 2 in which said effective amount of HMB is within the range from 0.01 to 0.20 grams of said HMB based on its calcium salt per kilogram body weight per 24 hours.

[*4] 4. The method of claims 1 or 2 in which said HMB is administered orally.

[*5] 5. The method of claims 1 or 2 in which said HMB is administered by intravenous infusion.

[*6] 6. The method of treating an adult human patient having a negative nitrogen balance, comprising orally or intravenously administering to the patient from 0.5 to 10 grams per 24 hours of beta -hydroxy- beta -methylbutyric acid (HMB) based on its calcium salt, said HMB being in an edible or intravenously-administrable form selected from (i) its free acid form, (ii) its sodium, potassium, magnesium, or calcium salt, (iii) its methyl or ethyl ester, or (iv) its lactone, and continuing the said administration of HMB until the loss of nitrogen is substantially reduced as determined by nitrogen analysis of the patient's urine.

[*7] 7. The method of claim 6 in which said HMB is in the form of its calcium salt (Ca-HMB).

[*8] 8. The method of claim 6 in which said HMB is in the form of its sodium salt (Na-HMB).

[*9] 9. The method of claims 6,7 or 8 in which said HMB is administered orally to the human patient in an amount of from 2 to 6 grams per 24 hours based

on its calcium salt.

[*10] 10. The method of claims 6, 7 or 8 in which said HMB is administered by intravenous infusion in an amount of 2 to 6 grams per 24 hours.

[*11] 11. The method of improving protein nutrition in elderly human subjects, comprising including in the daily diets of the human subjects an amount of from 0.5 to 10 grams per 24 hours of beta -hydroxy- beta - methylbutyric acid (HMB) based on its calcium salt, said HMB being in an edible form selected from (i) its free acid form, (ii) its sodium, potassium, or calcium salt, (iii) its methyl or ethyl ester, or (iv) its lactone, said amount of said HMB incorporated in said diets being effective to reduce the urinary nitrogen losses of the subjects.

[*12] 12. The method of claim 11 in which said HMB is in the form of its calcium salt (Ca-HMB).

[*13] 13. The method of claims 11 or 12 in which the amount of HMB incorporated in said daily diets is from 2 to 6 grams per 24 hours.

[*14] 14. The method of treating a human patient who is receiving an intravenous therapy and is subject to excessive loss of nitrogen, comprising incorporating in an intravenous solution being given to the human patient an effective amount of HMB for reducing urinary nitrogen, said HMB being in an intravenously administerable form selected from Ca-HMB or Na-HMB.

1 of 1 DOCUMENT

5,360,613

Nov. 1, 1994

Method of reducing blood levels of total cholesterol and
low-density lipoprotein cholesterol

LIT-REEX: NOTICE OF LITIGATION

Met-Rx Substrate Technology, Inc. v. Metabolic Technologies, Inc., et al,
Filed May 3, 1999, D.C. C.D. California, Doc. No. CV99-4766 ABC (VAPx)

INVENTOR: Nissen, Steven L., Ames, Iowa

ASSIGNEE-AT-ISSUE: Iowa State University Research Foundation, Inc., Ames,
Iowa (02)

APPL-N0: 136,688

FILED: Oct. 14, 1993

CERTCORR: Feb. 7, 1995 a Certificate of Correction was issued for this Patent

REL-US-DATA:

Continuation-in-part of Ser. No. 946,404, Sep. 16, 1992 now abandoned

INT-CL: [5] A61K 47#00

US-CL: 424#439; 424#451; 424#464; 514#824; 514#838; 514#893;

CL: 424;514;

SEARCH-FLD: 424#451, 439, 464; 514#824, 838, 893, 557

REF-CITED:

U.S. PATENT DOCUMENTS

3,629,449	12/1971	*	Siddiqi et al.	424#317
4,760,090	7/1988	*	Nissen	514#557
4,764,531	8/1988	*	Nissen	514#557
4,992,470	2/1991	*	Nissen	514#578
5,028,440	7/1991	*	Nissen	426#2
5,087,472	2/1992	*	Nissen	426#623

OTHER PUBLICATIONS

Tanaka et al., Biochim. Biosphys. Acta. 152:638-641 (1968).

Landass, Clin. Chim, Acta. 64:143-154 (1975).

Sabourin, Metabolism 32:160-164 (1983).

Mock et al., J. Lab. Clin. Med. pp. 240-247 (1988).

Van Koevering et al., Am. J. Physiol. 262:E27-E31 (1992).
Adamson et al., Biochem. Biophys. Acta. 23:4720479 (1957).
Yousufzai et al., Lipids, 11:526-529 (1975).
Lupien et al., J. Clin. Pharm. 19:120-126 (1979).
Gey et al., Helvetica Chim. Acta., 40:2354-2368 (1957).
Coffman et al., J. Am. Chem. Soc., 80:2882-2997 (1958).

PRIM-EXMR: Phelan, D. Gabrielle

LEGAL-REP: Tilton, Fallon, Lungmus & Chestnut

ABST:

Patients with elevated blood levels of low-density lipoprotein (LDL) and total cholesterol are treated by administering beta -hydroxy- beta -methylbutyric acid (HMB) to reduce the patient's blood level of LDL and total cholesterol. HMB can be safely administered orally to humans in amounts that will significantly reduce blood levels of total cholesterol and LDL.

NO-OF-CLAIMS: 6

EXMPL-CLAIM: 1

NO-OF-FIGURES: 0

NO-DRWNG-PP: 0

PARCASE: RELATED APPLICATION

This application is a continuation-in-part of copending application Ser. No. 07/946,404, filed Sep. 16, 1992, now abandoned.

SUM:

FIELD OF INVENTION

This invention relates to methods of reducing blood levels of cholesterol, and more particularly the reduction of low-density lipoprotein (LDL) cholesterol. The invention involves oral administration of a therapeutic agent for decreasing plasma cholesterol concentration.

BACKGROUND OF INVENTION

It is generally accepted that elevated blood cholesterol is a causative factor of coronary heart disease. Moreover, it is recognized that high blood levels of the form of cholesterol known as low-density lipoprotein (LDL) can contribute to cardiovascular disease. Guidelines have been established to indicate to doctors when patients should be considered at risk. Desirable is less than 200 mg/dl, borderline is 200 to 239 and high is greater than 140 mg/dl Schueker et al., Arch. Inter. Med. (1991) 151:666-673. If total cholesterol is greater than 240 mg/dl and/or LDH is above 160 mg/dl, therapeutic treatment may be needed. [See Goodman, Amer. J. Med., 90:2A-32S to 2A-35S (1991).]

The development of the present invention began with experiments conducted at Iowa State University, Ames, Iowa, U.S.A. in which metabolic products of leucine were feed to domestic animals. As described in U.S. Pat. No. 4,760,090 of

Steven L. Nissen, it was found that ketoisocaproic acid (KIC) can be feed to cattle and sheep for enhancement of growth and feed efficiency. It was observed that during such KIC feeding there was some reduction in plasma cholesterol, and also in the deposit of cholesterol in the meat. (See 4,760,090, col. 5-6, Table B.)

In another application of KIC feeding, egg production of laying chickens was increased, as described in U.S. Pat. No. 4,764,531 of Steven L. Nissen. It was found that the eggs of KIC feed chickens had reduced yolk cholesterol (4,764,531, col. 4, Table B).

In later experiments carried out by Dr. Steven L. Nissen at Iowa State University, beta -hydroxy- beta -methylbutyric acid (HMB) was fed to domestic animals. The effects obtained were different than with KIC. Metabolically, KIC and HMB are not equivalents. KIC is the only metabolic product of leucine, while HMB is a minor product of KIC metabolism.

Leucine is either used for protein synthesis in the body or is converted directly to KIC. In the mitochondria KIC is decarboxylated to isovalerylCoA and then further metabolized to ketone bodies. In certain disease conditions, such as isovaleric acidemia, an alternate oxidative pathway for KIC has been observed, which appears to produce beta -hydroxy- beta -methylbutyrate (HMB). In atypical cases, such as a genetic absence of the dehydrogenase enzyme, there is evidence that HMB can accumulate in the urine: Tanaka, et al. Biochim. Biosphys. Acta. 152:638-641 (1968). Also, in acidosis conditions, HMB levels can be increased in urine: Landass, Clin. Chim. Acta. 64:143-154 (1975). This presumably occurs by oxidation of KIC to HMB by the enzyme alpha ketoisocaproate oxygenase (Sabourin, Metabolism (1983) 32:160-164). Increased urine HMB can also occur in cases of biotin deficiency (Mock, J. Lab. Clin. Med. (1988) 240-247). The only evidence for normal HMB production is in lambs and pigs, Vankowering and Nissen, Am. J. Physiol. (1992) 262:E27-E31. In this study it was estimated that < 10% of leucine metabolic is via HMB production.

The differing activities of HMB as fed to domestic animals provided the basis for additional patents of Steven L. Nissen. His U.S. Pat. No. 4,992,470 discloses the administration of HMB for enhancing the immune response of mammals and as an ingredient in the raising of meat producing animals (e.g. ruminants and poultry) to increase lean tissue development. (See U.S. Pat. Nos., 5,087,472 and 5,028,440 of Steven L. Nissen.)

There has been a scientific effort to determine how cholesterol is synthesized in the bodies of mammals. It was known that acetate can be synthesized into cholesterol. Research investigations in the 1940's and 1950's concentrated on experiments with organic acids which also incorporated acetate and whose tracers could be incorporated into cholesterol. A small group of organic acids appeared to meet these qualifications. This included beta -hydroxy- beta -methylglutarate (HMG), beta -hydroxy- beta -methylbutyrate (3-hydroxy isovalerate), beta - beta -dimethylacrylate (DMA), isovalerate, and beta -methyl-gluconate (beta MG). ¹⁴C from ¹⁴C-acetate can be detected in all these compounds. Today it is thought that HMG-CoA is the obligatory precursor to cholesterol, and the other compounds referred to herein are somehow incorporated in cholesterol by interconversion with HMG. (Adamson et al. 1957, Biochem. Biophys. Acta, 23: 472-479.) Thus, although there is a biochemical relationship between HMG and HMB, it is not clear if there is any relationship between the compounds regarding effects on cholesterol metabolism.

Experiments demonstrated that feeding HMG to rats could decrease total serum

cholesterol by up to 20%. Effects on LDL cholesterol were not reported (Yousufzai et al., Lipids, 11:526-529).

Only limited human studies have been carried out with HMG. One study did measure the effect of HMB on subjects with familial hypercholesterolemia, and LDL was measured. A modest decrease in total cholesterol and LDL cholesterol was reported. (Lupien et al., J. Clin. Pharm., 19:120-126, 1979.)

After 8 weeks of being fed 3 grams of HMG daily, total cholesterol decreased from 404 to 353 mg % (- 13%) and LDL decreased from 333 to 307 mg % (- 8%). HDL cholesterol decreased approximately 35%. Thus, HMB appears to act differently from HMG in humans in that the effect is more pronounced and results in a specific decrease in LDL cholesterol but not in HDL cholesterol.

U.S. Pat. No. 3,629,449 claims that oral HMG can reduce serum cholesterol (total) and blood lipids (triglycerides) in warm-blooded animals.

Only one study is known where HMB was fed to animals, and an index of cholesterol metabolism measured: Gey et al., Helvetica Chim. Acta, 40:2354-2368 (1957). In that study HMB was fed to rats at a rate of 0.5 g/kg body weight for 2 and 4 days. At the end of the study, cholesterol synthesis was measured by removing the liver which was incubated in slices with ¹⁴C acetate. Cholesterol was isolated following the incubation and radioactivity quantitated. It was found that HMB had not significantly lowered the rate of acetate incorporation into cholesterol by the rat liver as compared to controls. In the same paper, an in vitro interaction of HMB and acetate incorporation was assessed. When HMB was added to the media at very high concentrations, it was found that there was no significant inhibition of acetate incorporation compared to the control values.

SUMMARY OF INVENTION

This invention is based on the development of scientific evidence that beta - hydroxy- beta -methylbutyric acid (HMB) can be used as an effective anti-cholesterol agent. In particular, the scientific data developed to date indicates that HMB can be safely administered orally to humans in amounts that will significantly reduce blood levels of total cholesterol, and, even more importantly, blood levels of low-density lipoprotein (LDL) cholesterol. HMB may be produced in small amounts from the amino acid leucine provided by protein-containing foods. However, administration of leucine or its metabolic conversion product ketoisocaproic acid (KIC) have not been reported to be effective anti-cholesterol agent in humans.

DETAILED DESCRIPTION

The compound used for practicing the present invention is beta -hydroxy- beta -methylbutyric acid (HMB), or edible derivatives thereof which directly convert in the body to HMB. The free acid compound is also called beta -hydroxy- isovaleric acid. It has the following structure: [See Original Patent for Chemical Structure Diagram]

While HMB can be administered in its free acid form, it is preferred to administer an edible form of HMB which is a salt, ester, or lactone. The calcium salt is preferred because it is less hygroscopic than the sodium or potassium salts, but those salts can also be used, depending on the mode of oral administration. Esters of HMB such as particularly the methyl or ethyl esters are also suitable. Such esters are rapidly converted in the body to the free acid form of HMB. For administration as a lactone, the compound isovaleryl lactone can be used. This compound and similar lactones are rapidly converted in

the body to free acid HMB.

The free acid form can be more specifically designated as "HMB acid". The salt forms, such as the calcium, sodium, potassium or magnesium salts, as "Ca-HMB", "Na-HMB", "K-HMB", and Mg-HMB. Correspondingly, the esters can be designated "HMB-methyl ester", "HMB-ethyl ester", etc. The lactone can be designated "HMB-lactone". HMB has no stereo-isomers and accordingly does not exist in L or D forms.

HMB is not currently commercially available. However, procedures are known for synthesizing this compound from commercially available starting materials. For example, HMB can be synthesized by oxidation of diacetone alcohol (4-hydroxy-4-methyl-2-pentanone). One suitable procedure is described by Coffman, et al., J. Am. Chem. Soc., 80:2882-2887, at 2885 (1958). As there described, beta -hydroxy-isovaleric acid (HMB) is synthesized by an alkaline sodium hypochlorite oxidation of diacetone alcohol. The product is recovered in free acid form, which can be converted to the desired salt. For example, HMB can be prepared as its calcium salt (Ca-HMB) by a similar procedure to that of Coffman, et al. in which the HMB acid obtained is neutralized with calcium hydroxide, and recovered by crystallization from an aqueous ethanol solution. For example, a 95% ethanol solution can be used with the Ca-HMB at about a 10% concentration.

Since Ca-HMB is a preferred form for administering HMB, the dosage amount of HMB can be expressed in terms of corresponding mole amount of Ca-HMB. The dosage range within which HMB can be usefully administered is from 0.01 to 0.2 grams HMB (Ca-HMB basis) per kilogram of body weight per 24 hours. For adults, assuming body weights of from about 100 to 200 lbs., the dosage amount of HMB (Ca-HMB basis) can range from 0.5 to 10 grams per patient per 24 hours. For most adults, on the basis of present data, it is believed that the optimum dosage is in the range from 2 to 6 grams of HMB (Ca-HMB) per 24 hours.

Ca-HMB and other forms of HMB as described above can be processed as fine powders which can be filled into capsules, or combined with tableting diluents, such as lactose, and compressed into tablets of predetermined dose amounts. No special mode of administration is needed. One preferred mode is to package the Ca-HMB in water-soluble capsules, such as gelatin capsules. Each capsule may contain as the predetermined amount of the Ca-HMB 0.5, 1, or 2 grams. Multiple doses per day are desirable, and therefore smaller dose sizes are believed preferable. However, if desired, larger doses in capsules or tablets can be prepared, such as 4 grams per capsule or tablet. A suitable regiment for oral administration to adults consists of one tablet or capsule one to four times per 24 hours. If taken once per day, it is preferred it be consumed before bedtime.

DETDESC:

The experimental basis of the present invention and the results that can be obtained can be more fully appreciated and understood from the following examples.

EXAMPLE I

A preliminary experiment was carried out using hamsters as the test animal and Na-HMB as the therapeutic agent. The experimental details and the results obtained are described as follows.

Pregnant Female hamsters were housed and allowed to give birth. At three weeks of age, litters were subdivided into pairs within a sex. The pairs of

hamsters were then assigned randomly a purified diet based on casein and cornstarch with either contained 0.1% NaCl (control) or one containing 0.1% Na-HMB (HMB). They were maintained on this diet for 6 weeks at which time they were killed by decapitation and blood collected into EDTA containing tubes. Blood was separated by centrifugation and plasma collected. The unfrozen plasma was treated with an LDL precipitating agent, centrifuged and the supernatant collected. The whole plasma and supernatant were assayed for cholesterol by an enzymatic method. Plasma cholesterol estimated total cholesterol while the supernatant represented HDL cholesterol. LDL cholesterol was estimated by the formula: LDL-Cholesterol = (Total cholesterol) - (HDL cholesterol) - (triglycerides X 0.2). Total triglycerides in plasma were estimated by an enzymatic assay. The results are summarized in Table A.

TABLE A
CHOLESTEROL (mg %)

Sex	Diet	No.	Total	HDL	LDL	HDL/ LDL	Trigly- cerides mg %
Fe- males	Con- trol	8	237	125	64	.51	240
Fe- males	HMB	8	228	132	53	.41	212
% Change			- 4%	+ 5%	- 17%	- 20%	- 11%
Males	Con- trol	7	217	111	59	.53	232
Males	HMB	7	208	118	45	.38	226
% Change			- 4%	+ 5%	- 24%	- 28%	- 2%

The foregoing data indicates that HMB can markedly lower LDL cholesterol in hamsters. The data also suggests a trend to increase HDL cholesterol, and that the ratio of LDL/HDL is positively affected.

EXAMPLE II

In view of the encouraging results of Example I, a large mammal study was carried out with lambs. HMB was administered in the form of Ca-HMB.
Animals and Feeding

Cross-bred lambs were obtained from a research flock. Males were selected from a pool of 108 rams while females were selected from a pool of 63 females. Selection was based on weight range, breed and previous performance. Breeding consisted of Dorset, Polypay and Suffolk crosses. Males were divided into 5 weight blocks and females into 3 weight blocks. The two heaviest male blocks contained 27 lambs, allotted to 3 pens. All other blocks contained 18 lambs, allotted to 2 pens. Animals were weighed on consecutive days prior to starting the experiment. All animals were shorn 100 days into the experiment. The animals were housed in a single confinement unit with uniform pens. The feeders were concrete bunks with water supplied by several nipple waterers per pen. Dirt flooring was bedded with oat straw when necessary. Weight blocks were placed in adjacent pens with treatment randomly assigned to the blocks. The ration was complete in all nutrients for growing sheep. The diet was formulated to contain protein in excess of the normal requirement in an effort to assure that protein was not a limiting factor for any growth response. The feeding schedule consisted of two feedings per day with the amount controlled so that the animals

had eaten all the feed from the previous feeding before being fed again. Also with the feed allotment, a top-dressing of 20 g of either a control premix containing HMB was added at the equivalent of 0.5 gram per animal per day and 1.5 gram per animal per day.

Preparation of HMB

Ca-HMB was prepared by minor modification of the method of Coffman, et al. J. Am. Chem. Soc., 80:2882-2887 (1958). More specifically, the crude HMB was first purified by distillation under vacuum, neutralized with $\text{Ca}(\text{OH})_2$, and finally the calcium salt crystallization three times from 95% ethanol. The product was then air-dried and fine-ground. Each batch was given a lot number and the purity assessed by high performance liquid chromatography. A single peak was measured when HMB was chromatographed on a C18 column and eluted with 0.01M phosphate buffer, pH 7.0. Also nuclear magnetic resonance was performed. This indicated only two peaks which corresponded to the methyl hydrogens and the CH_2 hydrogens.

Blood was collected from each animal. The plasma was analyzed for cholesterol using an Abbott Spectrum Diagnostic system. In all cases the pen means were used for analysis of variance. The general linear models procedure of the Statistical Analysis System (SAS) was used to analyze the model. A linear effect of HMB level was evaluated.

Results

The studies indicated that oral consumption of HMB decreased plasma cholesterol. The relevant data is summarized below in Table B.

TABLE B
Daily Consumption of HMB

	*	0.5 g	1.5 g	Linear
	Control	HMB	HMB	Effect
No. Pens	8	8	2	
Total animals	71	71	17	
Plasma cholesterol	54.3	51.3	48.1	0.05

EXAMPLE III

A further large mammal study was carried out with pigs. Pigs comprise test animals which are more similar in certain respects to humans than ruminants.

Feeding Regimens

Diet	*		*		Dose/kg body wt.
	Composition		Daily Dose		
Control:	60 g. of calcium carbonate per 227 kg of diet	0	*	0	
0.01% HMB	22 g of calcium HMB per 227 kg of diet	120	mg/day	1.5	mg/kg
0.05% HMB	113 g of calcium HMB per 227 kg of diet	1000	mg/day	12.5	mg/kg
0.05% KIC	113 g of calcium KIC per 227 kg of diet	1000	mg/day	80	mg/kg

The pigs were housed in pens of two animals each and were allowed ad libitum access to food and water. Pigs were approximately 160 lbs. at the start of the experiment and were 240 lbs. at the end. All pigs were castrate males. At day 28 and day 43 of the experiment blood was collected from the anterior vena cava by venipuncture. Blood was collected into EDTA containing tubes, centrifuged and frozen until analyzed. At approximately 100 days of the experiment half of the animals (32) were slaughtered, and at 120 days the remaining 32 animals were slaughtered. At the time of slaughter the abdominal aorta was dissected out and external fat removed. The aorta was then split lengthwise and 1/2 fixed in formaldehyde for 48 hours. At this time aortas were removed, stretched over a 6 inch stick, washed in ethanol and stained with a lipid stain for 2 hours. After a 1 hour wash aortas were blotted and examined. All the aortas from a group were laid out on a white bench and arranged in order from least severe to most severe. The least severe had no dark red streaks on the aorta or around the small vertebral vessels leaving the aorta. The most severe lesions had multiple streaks in the middle of the aorta and dark red deposits in turbulent areas such as the branching of vessels from the aorta. The ordered aortas were then assigned a consecutive decimal number. The first (least severe) was assigned 0 and the most severe was assigned 3.2 Plasma cholesterol and triglycerides were measured by an enzyme-colorimetric assay (Sigma).

Data Analysis and Results

statistical analysis was accomplished by the general linear model procedure of the Statistical Analysis System (SAS). The model included the main effects of treatment and pen number. T-tests were conducted from the ANOVA standard error, to compare the HMB treatments to the control. The feeding of HMB to swine indicated that cholesterol metabolism is altered by chronic feeding of HMB. HMB-fed pigs had lower deposits of fat in the aorta. It appeared that HMB can partially prevent the formation of pre-atherosclerotic lesions. No effect of KIC was noted relative to any parameter. The data is summarized below in Table C.

TABLE C

Variable	Cont.	Dietary HMB		KIC	Statistical Comparison		
		.01%	.05%		C vs. .01%	C vs. .05%	C vs. KIC
Aorta Streaking	1.87	1.23	1.18	1.48	.12	.06	ns
Plasma Cholesterol (mg %)	135	120	118	139	.14	.05	ns
Plasma Triglycerides (mg %)	107	105	109	106	ns	ns	ns

EXAMPLE IV

In view of the findings of the foregoing examples, a human study was carried out. HMB was administered at comparable doses to that used with the pigs and in the form of Ca-HMB.

Experimental Procedure

In a controlled double-blind study, the effects of feeding HMB on loss of urinary nitrogen, blood cholesterol and immune function in normal humans were

tested. This study also measured blood components that reflect liver, kidney and tissue metabolism. In addition further measurements of nitrogen metabolism and immune function were implemented. All measurements were made in a controlled dietary situation and under blinded conditions. Normal male subjects were used who had been screened for normalcy. Ca-HMB was administered in 250 mg capsules. The subjects were instructed to take the capsules in 4 equal doses daily (with meals and at bed time). The subject ate all their meals under controlled conditions. Normal diets were used, and the amount of the diets was controlled to maintain equal and substantially constant nitrogen intake. The subjects had blood drawn before the morning meal or before they took the morning HMB dose. Each subject was studied twice: once with a placebo and once with HMB. The subjects did not know which preparations they were given, LDL-cholesterol was calculated by the following formula: $\text{LDL-cholesterol} = (\text{Total Cholesterol}) - (\text{HDL-Cholesterol})$.

Results and Analysis

Plasma HMB in control subjects averaged 1.8 μM HMB while HMB treated averaged 12 μM . This increase in plasma HMB produced the results summarized in Table D.

TABLE D

	Control Group		Treated Group		% Change		% Net Effect
	Cont.<(a)>	Pla<(b)>	Cont.<(a)>	HMB<(c)>	Cont.	Trt.	
Weight (lbs.)	167	167	176	179	0	1.4	1.4
% Body fat (skin fold)	12.4	12.0	12.5	11.0	- 3	- 12	- 9
Resting metabolic rate	67.5	70.5	74.8	77.8	4	4	0
Total Cholesterol	172	166	187	169	- 3	- 10	- 6
HDL Cholesterol	49.5	45.6	49.5	50.9	- 8	3	11
LDL Cholesterol	113	118	118	107	2	- 8	- 10
LDL/HDL	2.28	2.58	2.38	2.10	13	- 12	- 25

n<a> no treatment -

n placebo treatment with CaCO_3 on same dose schedule and Ca level as Ca-HMB treatment -

n<c> Ca HMB treatment at 1.0 grams/24 hours -

n<d> % change from control to placebo and control to HMB treatment -

Body weight, body fat and resting metabolic rate (KCal/mw) did not change with HMB treatment. Consuming 2 g of HMB daily for two weeks reduced total cholesterol 6% and LDL cholesterol 10%, HDL cholesterol increased 11%. The ratio of LDL to HDL decreased 25% in two weeks. Because this study was only two week in duration, the maximum effect obtainable from HMB consumption was probably not obtained. The two week test period was too short. However, this study did show that HMB can effectively decrease LDL cholesterol and favorably change the LDL/HDL ratio in humans.

TABLE E
DAILY DOSAGE OF Ca-HMB

				* (PL)
% Change After 8 Days				Linear
0.5 g	1.0 g	2 g	4 g	Effect

Weight	1.7	- .3	.5	.2	.32
Body fat (%)	5	- 18	- 15	- 1	NS
Glucose	- 7	2	- 5	- 4	.99
Cholesterol	6	1	- 2	- 4	.21
LDL	2	0	- 3	- 6	.26
HDL	0	6	1	0	.85
Triglycerides	26	- 16	- 8	36	.50

EXAMPLE V

Higher dose levels than in Example IV of Ca-HMB were tested in humans. The objective was to obtain an indication of whether higher doses could be safely administered, and also whether higher doses might provide a greater reduction in total cholesterol and LDL cholesterol. The experimental procedure and results obtained are summarized below.

Experimental Procedure

Normal humans were used in the study. Ca-HMB was administered in 250 mg capsules, and the subjects were instructed to take the capsules in 4 equal doses daily. No control of diet was attempted except they were instructed to eat as they normally would. On alternate days the subjects had blood drawn before the morning meal and before they took the morning HMB dose. Four subjects were studied for dose level of 0.5, 1, 2 and 4 grams per day.

Analysis

Results from all samples of each subject were subjected to linear regression and the slope and intercept used to calculate the % change in concentration after 8 days on treatment. The percentage changes were then subjected to ANOVA-regression to determine if there was a linear effect of dosage on the parameter. The dose-response effect ($p <$) is listed in the linear effect column of Table E.

Discussion Of Results

Blood HMB increased in a dose-responsive manner. A very large increase in blood HMB occurred at 4 grams per day. This suggests that higher doses than the 1 g per day of Example IV are desirable for maximum cholesterol reduction. In general there were no adverse affects of HMB noted. The only complaint noted with several patients was of being hungrier than normal. Body fat measurements were somewhat limited in this study in that only half the subjects had the measurements. However, the study combined with the study of Example IV is extremely suggestive of an effect on body fat. Over the course of the week, blood cholesterol decreased in a dose-responsive manner as shown in Table E. At an HMB dose of 4 grams per day, total cholesterol and LDL cholesterol decreased about 6% by the end of the one week study. An HMB dose of 2 grams daily also appeared to decrease cholesterol, but at lower doses no effect was clearly observable in this short term study.

CLAIMS: I claim:

[*1] 1. The method of treating a human patient having elevated blood levels of low-density lipoprotein cholesterol and total cholesterol, comprising orally administering to the human patient an effective amount of an edible form of beta-hydroxy- beta -methylbutyric acid for reducing the patient's blood level of said lower density cholesterol and total cholesterol, said edible form consisting of (i) its free acid form, or (ii) its sodium, potassium, or calcium

salt, or (iii) its methyl or ethyl ester, or (iv) its lactone, said effective amount being within the range of from 0.01 to 0.20 grams of said edible form per kilogram body weight per 24 hours based on said calcium salt.

[*2] 2. The method of claim 1 in which said edible form is its calcium salt.

[*3] 3. The method of claims 1 or 2 in which said effective amount of said edible form is from 0.01 to 0.20 grams per kilogram body weight per 24 hours based on its calcium salt.

[*4] 4. The method of treating a human patient having an elevated blood level of low-density lipoprotein cholesterol and total cholesterol, comprising orally administering to the human subject an edible form of beta -hydroxy- beta -methylbutyric acid to reduce the patient's blood level of said low density cholesterol and total cholesterol, said edible form consisting of (i) its free acid form, or (ii) its sodium, potassium, magnesium, or calcium salt, or (iii) its methyl or ethyl ester, or (iv) its lactone said edible form being administered in an effective amount within the range from 0.5 to 10 grams per 24 hours based on said calcium salt.

[*5] 5. The method of claim 5 in which said HMB is in the form of its calcium salt (Ca-HMB).

[*6] 6. The method of claim 4 or claim 5 in which said HMB (Ca-HMB basis) is administered to the human patient in an amount of from 2 to 6 grams per 24 hours.

1 of 1 DOCUMENT

5,087,472

Feb. 11, 1992

Feed compositions for domestics animals containing
hydroxymethylbutyrate

INVENTOR: Nissen, Steven L., Ames, Iowa

DISCLAIMER: Feb. 12, 2008

ASSIGNEE-AT-ISSUE: Iowa State University Research Foundation, Inc., Ames,
Iowa (02)

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5,028,440

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US-CL: 426#623; 426#2; 426#630; 426#636; 426#807; 514#557

CL: 426;514

SEARCH-FLD: 426#635, 807, 2, 623, 630, 656, 636; 514#557

REF-CITED:

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PRIM-EXMR: Penland, R. B.

LEGAL-REP: Tilton, Fallon Lungmus & Chestnut

ABST:

Feed compositions are provided for raising meat-producing ruminants and polutry. beta -hydroxycy- beta -methylbutyric acid or an edible salt thereof (HMB) is incorporated in the ruminant or poultry feeds to obtain a substantial increase in meat and/or to improve the quality of the lean meat.

NO-OF-CLAIMS: 2

EXMPL-CLAIM: 1

NO-OF-FIGURES: 0

NO-DRWNG-PP: 0

PARCASE: RELATED APPLICATION

This application is a continuation-in-part of copending application Ser. No. 07/472,090, filed Jan. 30, 1990 U.S. Pat. No. 5,028,440.

SUM:

BACKGROUND OF INVENTION

The keto analog of L-leucine (a dietarily essential amino acid) is o-keto-isocaproic acid, which is usually referred to as ketoisocaproate (KIC), or

sometimes also as ketoleucine. In the accepted description of leucine metabolism, leucine is first transaminated to its ketoacid, alpha - ketoisocaproate (KIC). KIC then enters the mitochondria and is decarboxylated to isovalerylCoA by the branched chain ketoacid dehydrogenase. [See Krebs, et al., Adv. Enz. Reg. 15:375-394 (1976); and Paxton, et al., J. Biol. Chem. 257:14433-14439 (1982).] An alternate minor pathway has been described in the rat and human liver [Sabourn, et al., Fed. Proc. 38:283 (1979)]. This alternate oxidative pathway occurs in the cytosol and involves oxidation of KIC to beta - hydroxy- beta -methyl butyrate (HMB) by the enzyme KIC-oxygenase [Sabourn, et al., Arch. Biochem. Biophys. 206:132-144 (1981)].

The administration of keto analogs of amino acids has been proposed for treatment of certain disease conditions in humans, such as uremia. [See, for example, Walser, et al., J. Clin. Inv., 52:678-690 (1973).] For nutritional purposes, it is known that KIC is an inefficient substitute for leucine. Rat studies have shown that the feeding of KIC as a replacement for leucine requires the feeding of from two to three times as much KIC as the nutritionally required amount of leucine: Chawla, et al., J. Nutr., 105:798-803 (1975); and Boebel, et al., J. Nutr., 112:1929-1939 (1982); and Chow, et al., J. Nutr., 104: 1208-1214 (1974).

It has been proposed to feed small amounts of KIC in conjunction with animal diets containing sufficient leucine for the purpose of improving the growth metabolism of the animals. By using milligram amounts of KIC, some increases in the rates of weight gain and/or feed efficiencies have been obtained. [See, for example, U.S. Pat. Nos. 4,760,090 and 4,883,817.] With mature sheep being fed for wool production, by feeding KIC the amount of wool produced may also be increased (U.S. Pat. No. 4,760,090). When lactating domestic animals, such as dairy cattle, are fed small amounts of KIC, the quantity of milk produced may be increased (U.S. Pat. No. 4,758,593). Egg production by laying chickens can also sometimes be increased (U.S. Pat. No. 4,760,531). Other effects of feeding KIC have been observed, including cholesterol reduction in meat, milk and eggs (U.S. Pat. Nos. 4,760,090 and 4,760,531), and some apparently beneficial effects on the immune system (U.S. Pat. No. 4,835,185).

Why KIC addition to protein and leucine sufficient animal diets can have different effects than the metabolic conversion of leucine to KIC is not known. The beneficial effects of KIC on domestic animals are not consistently obtainable. Variability of benefits have been particularly observed in the KIC feeding of ruminants. KIC supplementation has not become an established animal feeding practice for any purpose. With reference to the use of KIC in feeds for cattle and sheep, a major problem is that the KIC is subject to partial rumen destruction, which is a variable factor. The use of rumen-protective agents and procedures for KIC has not been shown to be satisfactory for commercial purposes.

Nutritionally, as described above, leucine is converted to KIC, which in the mitochondria is decarboxylated to isovalerylCoA. In certain disease conditions, such as isovaleric acidemia, an alternate oxidative pathway for KIC has been observed in the liver, which may produce the substance beta -hydroxy- beta -methylbutyrate (HMB). To date, however, there is little or no evidence that HMB is normally produced in the metabolism of KIC. In some extreme cases, such as genetic absence of the dehydrogenase enzyme, there is evidence HMB accumulates in the urine: Tanaka, et al., Proc. Natl. Acad. Sci., 56:236-242 (1966); and Tanaka, et al., Biochim. Biophys. Acta. 152:638-641 (1968). In acidosis conditions HMB levels may be increased in urine: Landass, Clin. Chim. Acta,

Animals can be stimulated to grow in a general way which increases all organs and tissues. In that case, overall weight gain is usually increased, and feed efficiency can also be increased, although usually not as much as the rate of gain. Muscle or lean tissue can be stimulated to grow at the expense of fat or other organs. In that case, weight gain rate may not change, but usually feed efficiency is improved. Thus, growth of lean tissue can be markedly stimulated without a major change in average daily gain, or other measure based on body weight. The meat industry is now moving toward paying producers on the basis of lean tissue weight rather than on total animal weight. Thus, it is becoming important to base performance on lean tissue gain and lean tissue feed efficiency. However, previous research with KIC had not shown that it specifically stimulates lean tissue gain (e.g., muscle growth). In some cases KIC has been shown to decrease fat deposition, such as in sheep, but KIC generally has not been found to stimulate muscle growth.

SUMMARY OF INVENTION

beta -hydroxy- beta -methyl butyrate (HMB) has been found to be more effective for improving growth metabolism of domestic animals than alpha - ketoisocaproate (KIC). A major effect of HMB is to increase markedly the development of lean tissue. This desirable effect is not obtainable with KIC although there is a slight tending for KIC to reduce the accumulation of body fat.

With reference to ruminants, HMB has the advantage of not being subject to appreciable rumen destruction. Consequently, HMB can be administered orally in defined amounts to ruminants, such as an additive to feeds for cattle or sheep, to obtain the growth metabolism effects and especially to increase the amount of lean tissues produced. Advantageous growth metabolic effects can also be obtained by the feeding of HMB to non-ruminants, including poultry being raised for meat production.

Increase in lean tissue by HMB feeding is typically accompanied by a decrease in the amount of fat contained in separate fatty layers, as distinguished from marbling fat distributed in lean tissue. With respect to beef cattle and sheep, feeding requirements take into consideration the distribution of fat (marbling) in the lean tissue, for example, by rib-eye examination. The feed compositions of this invention do not result in downgrading of carcasses because of reduced marbling, but instead provides as good or better fat distribution within the lean tissues. Juicy, flavorful lean meat can thereby be produced.

The desired growth effects described above can be obtained by the oral administration of calcium-HMB (Ca-HMB) or equivalent molar amount of other water-soluble non-toxic HMB salt. Amounts as low as 15 to 35 milligrams (mg) per kilogram (kg) of body weight per 24 hours can be used.

DETDESC:

DETAILED DESCRIPTION

The compound used for practicing the present invention is beta -hydroxy- beta -methyl butyric acid or an edible butyrate salt thereof. The free acid compound is also called beta -hydroxy-isovalaryic acid. It has the following structure:
[See Original Patent for Chemical Structure Diagram]

This compound in both free acid and salt form is referred to herein

generically as "HMB". The acid form is designated HMB acid, and the specific salt form, such as the calcium or sodium salts, as Ca-HMB or Na-HMB. HMB has no isomers and accordingly does not exist in L or D forms. For the purpose of the present invention, it is preferred to employ HMB in the form of an edible salt rather than as the free acid. Preferably the salt form is water-soluble or becomes water-soluble in the stomach or intestines of the domestic animal. A preferred salt is the calcium salt (Ca-HMB). Sodium (Na-HMB) can also be used but Na-HMB is more hygroscopic than Ca-HMB. Other non-toxic salts can be used such as other alkali metal or alkaline earth metal salts. For mixing with feed ingredients, it is preferred that the salt form be dry, non-sticky, and finely-divided for blending with the feed materials. Ca-HMB is particularly desirable for these reasons.

HMB is not known to be commercially available. However, procedures are known for synthesizing this compound from commercially available starting materials. For example, HMB can be synthesized by oxidation of diacetone alcohol (4-hydroxy-4-methyl-2-pentanone). One suitable procedure is described by Coffman, et al., J. Am. Chem. Soc., 80:2882-2887, at 2885 (1958). As there described, beta -hydroxy-isovaleryic acid (HMB) is synthesized by an alkaline sodium hypochlorite oxidation of diacetone alcohol. The product is recovered in free acid form, which can be converted to the desired salt. For example, HMB can be prepared as its calcium salt (Ca-HMB) by a similar procedure to that of Coffman, et al. in which the HMB acid obtained is neutralized with calcium hydroxide, and recovered by crystallization from an aqueous ethanol solution. For example, a 95% ethanol solution can be used with the Ca-HMB at about a 10% concentration. Such a procedure is illustrated in more detail in the following examples.

To assure administration at a desired level, it is preferred to mix the dry HMB salt with the dry feed ingredients to a predetermined concentration. The HMB salt can be incorporated by dry blending using standard mixing equipment. The HMB should be substantially uniformly distributed throughout the feed. After mixing, if desired, the feed material may be further processed, such as by conversion to pellets.

Most feed compositions for domestic animals, such as for the raising of ruminants or poultry, are composed of mixtures of feed ingredients. These feed compositions contain protein-providing ingredients as principal components. These feed ingredients usually provide at least 10% protein by weight on a total dry matter basis, and may contain as much as 24% or more by weight. Such mixed feed compositions may comprise complete feeds or feed concentrates.

When HMB is combined with the feed material as a uniform mixture, and the feed composition provides the major food source for the diet, the amount of HMB may be specified in relation to the feed composition. For example, the admixed total ration feed compositions may contain from 0.001 to 0.5 wt % HMB (Ca-HMB and dry feed basis). On the same basis, a presently preferred range is from about 0.01 to 0.1 wt. % HMB. Such complete feed compositions will usually contain at least 10% protein and may contain up to 24% protein (N x 6.25). For example, beneficial growth effects such as enhanced lean tissue development can be obtained in preferred embodiments by incorporating from about 0.02 to 0.04 wt. % (dry basis) of Ca-HMB or molar equivalent amount of another edible non-toxic water-soluble salt of HMB.

The foregoing feed composition levels of HMB relate primarily to feeds which are formulated to comprise substantially the total ration of the animal. Where feed supplements or feed concentrates are employed as the vehicle for administering the HMB, greater concentrations may be required. The

concentrations can be similarly related to either the total diet of the animal, or to the body weight of the animals being fed.

It is believed that some beneficial effects can be obtained with as little as 0.05 to 0.2 milligrams (mg) of HMB (Ca-HMB basis) per kilogram (kg) body weight per 24 hours. It will usually be desirable, however, to administer at least 0.5 mg/kg body weight/24 hrs (Ca-HMB basis). It will not usually be necessary to administer more than 100 mg HMB (Ca-HMB basis) per kilogram of body weight per 24 hours, but higher amounts can be given up to 400 to 500 milligrams HMB on the stated basis. An optimum range for most domestic animals is believed to be from about 15 to 35 mg/kg body weight/24 hrs (Ca-HMB basis).

As previously indicated, it is preferred to combine HMB with a complete feed ration, feed concentrate, or other dry feed material being given to the cattle, sheep, chickens, turkeys, or other domestic animal responsive to HMB. In certain cases, however, HMB could be administered by dissolving it in drinking water for the animals. However, control of the amount administered in water can be expected to be more difficult. For more precise control, HMB could be orally administered in the form of pellets. For example, such pellets could be spread as a top dressing on the daily (24 hr) feed ration for each animal. Other methods of administration could be used.

As far as is known, HMB is not subject to significant rumen destruction. Following oral administration, the HMB salt appears to pass intact through the rumen into the intestines of the ruminant where it is absorbed. For the purpose of the present invention, it is believed that HMB should be absorbed into and distributed in the circulatory system.

In improving growth metabolism of domestic animals, several different effects can be selected as a primary purpose of the HMB administration. For example, with respect to animals being raised for meat production, including beef cattle, such as steers and heifers, as well as lambs, and chicks, a principal objective can be to increase the development of lean tissue while minimizing the accumulation of body fat. Another objective is to improve the quality of meat by better fat distribution (marbling) in the lean meat as distinguished for separate accumulations of fat. Juicier and more flavorful meat can thereby be produced.

The method of this invention and the feed compositions for use therein can be further illustrated by the following experimental examples.

EXAMPLE 1

PREPARATION OF Ca-HMB

Reaction is contained in a 5000 ml round bottom flask fitted with condenser. Constant mixing is achieved by a magnetic stirrer.

Add in sequence:

1 gallon bleach (5.25% Sodium Hypochlorite)

45 g NaOH powder

Mix well.

150 ml 1,4 Dioxane

93 ml 4-Hydroxy-4-Methyl-2-Pentanone.

Reflux for 40 minutes.

Transfer solution to washtub and cool for 30 minutes under a hood.

pH to 5 using concentrated H₂SO₄. (HMB is stabilized.)

Transfer solution to cooling pans under a vent hood, so that air is drawn in over the solution for faster evaporation. Use the steam table if possible.

Let solution evaporate overnight. Sodium sulfate salts will precipitate out forming a slurry.

Transfer slurry to washtub, and adjust pH to 1 using concentrated H₂SO₄.

Salts and solution may be extracted separately depending on the volume available after evaporation.

Transfer solution with/without salts to 10 liter bottle, and wash 4 x with approximately 2 liters of Ethyl Acetate. (Acetate layer contains HMB.)

Save ethyl acetate layer. Discard final acid layer.

Roto Vap Acetate layer at 50o C.

Salts may precipitate out.

Solubilize in Ultra-pure H₂O.

Add an equal volume of Ethyl Acetate and re-extract. Save ethyl acetate layer.

Roto Vap Acetate layer at 50o C.

Increase Temperature to 70o C. and continue Roto Vap. Remaining solution contains HMB and is ready for crystallization.

The dried HMB acid is neutralized with calcium hydroxide powder. The powder is added to the stirring HMB acid until a basic pH is reached. The pH is assessed with pH paper. The HMB solidifies at this point and is subsequently dissolved in hot 95% ethanol at a volume of 10 times the original acid volume. Material that does not dissolve is removed from the liquid by centrifugation or filtration. The ethanol-HMB solution is then placed at - 20° F until the mixture crystallizes. Usually this takes overnight but can take 2-3 days. The HMB crystals are then filtered under vacuum through paper towels and liquid squeezed out of the cake-like crystals. The HMB crystals are then redissolved in hot ethanol and the process repeated. In most cases 3 recrystallizations are sufficient to fully remove any yellow color from the crystals. Further purification can be achieved by further crystallization. After the final crystallization the HMB is placed in a pan and freeze-dried overnight to obtain an anhydrous calcium HMB powder ready for feed additive use.

EXAMPLE 2

STABILITY OF HMB IN THE RUMEN

Rumen fluid was collected from a fistulated steer. After filtration and dilution (1:4) with an artificial saliva, 25 ml of the solution was added to 50 ml plastic tubes. Each tube was fitted with a one-way valve to allow gases to escape while not allowing air into the tube. Each tube was then gassed with CO₂ and incubated at 39o C. After 30 min a solution of KIC or HMB was added to the tube in concentrations to simulate what would be present in the rumen of an animal consuming 0.05% of the diet as KIC or HMB. It was estimated that a 50 mu M concentration would be attained in this case. At timed intervals 50 mu l samples of this rumen fluid were taken and analyzed for KIC and HMB. The results are shown in Table A.

TABLE A

Time after Addition (min)	* KIC	HMB
------------------------------	----------	-----

0	30*	60
15	15	76
30	4	81
60	2	71
240	2	74
480	1	74

n*Initial concentration of KIC could only be estimated. The initial concentrations should have been - 50 uM but because of the rapid degradation of KIC in the rumen, KIC was already being degraded before the 0 time collection could be cooled and processed. -

The foregoing shows that KIC is rapidly destroyed by the rumen bacteria while HMB is quite stable in a rumen environment.

EXAMPLE 3

FEEDING OF HMB TO LAMBS-TRIAL 1

Material and Methods

Animals and Experimental Design. Three sets of twin lambs were housed individually in fiberglass pens. Animals were randomly assigned to control or HMB diets, using Ca-HMB as the feed additive at a level of 0.5% of the basal diet.

TABLE B
Composition of Basal Diet Fed to All Sheep.

Ingredient	Kg Dry Matter Experiment 1
Ground corn	84.4
Expeller soybean meal	5.1
Molasses	6.5
Trace mineral & vitamin premix	0.66
Salt	0.66
Limestone	2.1
Urea	0.66

The results are summarized below in Table C.

TABLE C			
	Renal Fat % (% of animal live weight)	Back Fat (in) (fat thickness over 12th rib)	Longissimus area (sq. in.)
Controls:	2.42	.21	2.1
HMB:	1.90	.12	2.3
% change due to HMB	- 22%*	- 43%*	+ 10%
Average daily gain (kg/day)			
Feed/Gain			
Controls:	.287		3.89
HMB:	.296		3.60
% change due to HMB	+ 3%		- 7%

n*P < 0.5 -

As shown by the foregoing data, in this limited study HMB appeared to stimulate growth of lean tissues at the expense of fat. Both measures of fat decreased dramatically while the muscle weight increased substantially from controls.

EXAMPLE 4

FEEDING OF HMB TO LAMBS-TRIAL 2

Animals and Experimental Design. Twenty-two lambs were housed individually in fiberglass pens (1.15 m²) and randomly assigned to control or HMB diets. The control had nothing added to the diet while the HMB animals had 1 g of CaHMB supplemented to their diets each day. At the beginning of the experiment animals were weighed and ultrasound measurements made of the longissimus area and the amount of fat over the longissimus muscle. These measurements were made over the 12th rib area. Feed consumption was monitored. At the end of the experiment animals were weighed, feed consumption measured and the longissimus area and fat thickness measured again with the ultrasound apparatus. The gain in longissimus area and backfat were calculated from the initial value. This noninvasive method of estimating muscle and fat deposition has been shown to be a reliable method of assessing these values without slaughtering the animal.

TABLE D
Composition of Basal Diet Fed to All Sheep

Ingredient	Kg Dry Matter	
	Without HMB	With HMB
Ground corn	48.2	48.2
Dehydrated alfalfa	20.0	20.0
Expeller soybean meal	25.0	25.0
Molasses	5.0	5.0
Trace mineral & vitamin premix	0.2	0.2
Salt	0.5	0.5
Limestone	0.93	0.88
Ammonium chloride	0.30	0.30
Ca-HMB	0	0.050
Results:		

TABLE E
The Effects of Feeding HMB to Young Lambs
from 60 lbs. to 100 lbs.

	Control	HMB	% Change
Total gain (lbs)	38.5	40.5	+ 6%
Feed/gain	4.41	4.28	- 6%
Loin area gain (cm ²)	2.08	3.34	60%**
Back fat gain (cm)	.27	.19	- 30%**

n**P < .02 level -

The foregoing data indicates that HMB supplementation increases performance

and increases lean vs. fat gain. These results are therefore corroborative of those of the first trial.

EXAMPLE 5

FEEDING HMB TO POULTRY

To compare KIC and HMB as growth promotants, young male leghorn chicks were allotted to 12 battery (wire mess floors) pens consisting of 10 birds each. Treatments of control, HMB (0.04% of the diet) and KIC (0.04% of the diet) were assigned to the pens. Feed consumption was measured during the 5 week experiment. During week 2 of the experiment chicks were injected with 50 μ l of a 20% suspension of pig red blood cells intraperitoneally.

Chicks were fed a diet adequate in all nutrients for leghorns of this age. HMB was a supplement to this diet at 0.04% of the diet (w/w). The following diet was used as shown in Table F.

TABLE F

Ingredients (KG Each)	Control Diet	HMB Diet
Ground corn	59.3	59.3
Soybean meal	19.7	19.7
Wheat Middlings	13.4	13.4
Fish Meal	3.0	3.0
Calcium Carbonate	0.71	0.66
Dicalcium Phosphate	0.38	0.38
Meat and bone meal	3.0	3.0
Premix of vitamins & minerals	0.6	0.6
HMB	0.0	0.05

At the end of the 5 week experiment chicks were decapitated and serum collected for pig red blood cells antibody titer determination. Titers to red blood cells were evaluated by a microtiter agglutination test (Kuhlman, et al.). Also, the Pectoralis major muscle (the major breast muscle) was dissected and weighed as a measure of muscle growth. The data is summarized in Tables G1 and G2.

TABLE G1

Growth and Feed Efficiency of Male Leghorns
Fed KIC and HMB (5 week data)

	Control	KIC	HMB
Gain (g)	222	229	229
Feed/gain	2.30	2.24	2.17
Breast muscle (%)	2.84	2.94	3.00
Red blood cell titer (dilution)	1.57	2.65	3.25

TABLE G2

	KIC Change from Control	HMB Change from Control
Gain (g)	3.3%	3.3%
Feed/gain	- 2.5%	- 5.8%**
Breast muscle (%)	3.5%	5.5%**
Red blood cell titer (dilution)	68%	107%**

n**Significant at the $p < .02$ level -

The foregoing data shows that HMB increased performance of growing chicks by increasing the rate of growth and by making more efficient use of feed for growth. Additionally, it appears that muscle growth is stimulated relative to other tissues as evidenced by increased breast muscle size. Compared to KIC, HMB was superior in all parameters measured.

Feeding Periods

To obtain the advantages of the method and feed compositions of this invention for selectively increasing lean tissue formation while minimizing fat deposition in the raising of meat producing animals, it will be preferred to feed the animals the HMB on a daily basis while the animals are increasing in weight, and to continue the feeding for at least 10 days and up to 180 days, depending on the animals being fed. With beef cattle, for example, HMB is preferably fed for periods of at least 30 up to 180 days; with lambs, for periods of at least 15 up to 100 days; for chickens, for periods of at least 10 up to 50 days, and for turkeys, for periods of at least 10 up to 100 days. The periods referred to are when animals being raised are growing to market weight sizes. The maximum advantage of HMB administration should be obtained when fat being deposited and time deposition have decreased, which usually occurs in the later portion of feeding before marketing. Incorporation of HMB in finishing rations is therefore especially desirable.

CLAIMS: I claim:

[*1] 1. A feed composition for growing meat-producing beef cattle and lambs, consisting essentially of a protein-containing ruminant feed containing in admixture therewith from 0.01 to 0.1 weight percent of an edible salt of beta -hydroxy- beta -methylbutyric acid (HMB) on a Ca-HMB dry weight basis,

wherein said feed composition increases lean tissue development in the meat production of said beef cattle and lambs.

[*2] 2. A feed composition for growing meat-producing poultry, consisting essentially of a protein-containing poultry feed containing in admixture therewith from 0.01 to 0.1 weight percent of an edible salt of beta -hydroxy- beta -methylbutyric acid (HMB) on a Ca-HMB dry weight basis,

wherein said feed composition increases lean tissue development in the meat production of said poultry.

1 of 1 DOCUMENT

5,028,440

Jul. 2, 1991

Method of raising meat producing animals to increase lean
tissue development

INVENTOR: Nissen, Steven L., Ames, Iowa

DISCLAIMER: Feb. 12, 2008

ASSIGNEE-AT-ISSUE: Iowa State University Research Foundation, Inc., Ames,
Iowa (02)

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CL: 426;514

SEARCH-FLD: 426#2, 623, 630, 636, 807; 514#557

REF-CITED:

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PRIM-EXMR: Penland, R. B.

LEGAL-REP: Tilton, Fallon, Lungmus & Chestnut

ABST:

A method is provided for raising meat producing domestic animals to increase lean tissue development. beta -hydroxycy- beta -methylbutyric acid or an edible salt thereof (HMB) is administered to the animals in an effective amount for a sufficient length of time to obtain a substantial increase in lean tissue weight. Feed compositions for use in practicing the method are also provided. The method and feed compositions are particularly adapted for use with ruminants, including beef cattle and lambs, since HMB is not subject to appreciable rumen destruction. The method can also be practiced with other domestic animals, including chickens, and turkeys.

NO-OF-CLAIMS: 11

EXMPL-CLAIM: 1

NO-OF-FIGURES: 0

NO-DRWNG-PP: 0

SUM:

BACKGROUND OF INVENTION

The keto analog of L-leucine (a dietarily essential amino acid) is alpha - keto-isocaproic acid, which is usually referred to as ketoisocaproate (KIC), or sometimes also as ketoleucine. In the accepted description of leucine metabolism, leucine is first transaminated to its ketoacid, o-ketoisocaproate (KIC). KIC then enters the mitochondria and is decarboxylated to isovalerylCoA by the branched chain ketoacid dehydrogenase. [See Krebs, et al., Adv. Enz. Reg. 15:375-394 (1976); and Paxton, et al., J. Biol. Chem. 257:14433-14439 (1982).] An alternate minor pathway has been described in the rat and human liver [Sabourin, et al., Fed. Proc. 38:283 Abs. 312 (1979)]. This alternate oxidative pathway occurs in the cytosol and involves oxidation of KIC to beta -hydroxy-beta -methyl butyrate (HMB) by the enzyme KIC-oxygenase [Sabourin, et al., Arch. Biochem. Biophys. 206:132-144 (1981)]. According to Sabourin et al. (1981, p. 143): "The importance of this pathway is presently unknown".

The administration of keto analogs of amino acids has been proposed for treatment of certain disease conditions in humans, such as uremia. [See, for example, Walser, et al., J. Clin. Inv., 52:678-690 (1973).] For nutritional purposes, it is known that KIC is an inefficient substitute for leucine. Rat studies have shown that the feeding of KIC as a replacement for leucine requires the feeding of from two to three times as much KIC as the nutritionally required amount of leucine: Chawla, et al., J. Nutr., 105:798-803 (1975); and Boebel, et al., J. Nutr., 112: 1929-1939 (1982); and Chow, et al., J. Nutr., 104: 1208-1214 (1974).

It has been proposed to feed small amounts of KIC in conjunction with animal diets containing sufficient leucine for the purpose of improving the growth metabolism of the animals. By using milligram amounts of KIC, some increases in the rates of weight gain and/or feed efficiencies have been obtained. [See, for example, U.S. Pat. Nos. 4,760,090 and 4,883,817.] With mature sheep being fed for wool production, by feeding KIC the amount of wool produced may also be increased (U.S. Pat. No. 4,760,090). When lactating domestic animals, such as dairy cattle, are fed small amounts of KIC, the quantity of milk produced may be increased (U.S. Pat. No. 4,758,593). Egg production by laying chickens can also sometimes be increased (U.S. Pat. No. 4,760,531). Other effects of feeding KIC have been observed, including cholesterol reduction in meat, milk and eggs (U.S. Pat. Nos. 4,760,090 and 4,760,531), and some apparently beneficial effects on the immune system (U.S. Pat. No. 4,835,185).

Why KIC addition to protein and leucine sufficient animal diets can have different effects than the metabolic conversion of leucine to KIC is not known. The beneficial effects of KIC on domestic animals are not consistently obtainable. Variability of benefits have been particularly observed in the KIC feeding of ruminants. KIC supplementation has not become an established animal feeding practice for any purpose. With reference to the use of KIC in feeds for cattle and sheep, a major problem is that the KIC is subject to partial rumen destruction, which is a variable factor. The use of rumenprotective agents and procedures for KIC has not been shown to be satisfactory for commercial purposes.

Nutritionally, as described above, leucine is converted to KIC, which in the mitochondria is decarboxylated to isovalerylCoA. In certain disease conditions, such as isovaleric acidemia, an alternate oxidative pathway for KIC has been observed in the liver, which may produce the substance beta -hydroxy- beta -methylbutyrate (HMB). To date, however, there is little or no evidence that HMB is normally produced in the metabolism of KIC. In some extreme cases, such as genetic absence of the dehydrogenase enzyme, there is evidence HMB accumulates in the urine: Tanaka, et al., Proc. Natl. Acad. Sci., 56:236-242 (1966); and Tanaka, et al., Biochim. Biophys. Acta, 152:638-641 (1968). In acidosis conditions HMB levels may be increased in urine: Landass, Clin. Chim. Acta, 64:143-154 (1975).

Animals can be stimulated to grow in a general way which increases all organs and tissues. In that case, overall weight gain is usually increased, and feed efficiency can also be increased, although usually not as much as the rate of gain. Muscle or lean tissue can be stimulated to grow at the expense of fat or other organs. In that case, weight gain rate may not change, but usually feed efficiency is improved. Thus, growth of lean tissue can be markedly stimulated without a major change in average daily gain, or other measure based on body weight. The meat industry is now moving toward paying producers on the basis of lean tissue weight rather than on total animal weight. Thus, it is becoming important to base performance on lean tissue gain and lean tissue feed efficiency. However, previous research with KIC had not shown that it specifically stimulates lean tissue gain (e.g., muscle growth). In some cases KIC has been shown to decrease fat deposition, such as in sheep, but KIC generally has not been found to stimulate muscle growth.

SUMMARY OF INVENTION

beta -hydroxy- beta -methyl butyrate (HMB) has been found to be more effective for improving growth metabolism of domestic animals than o - ketoisocaproate (KIC). A major effect of HMB is to increase markedly the

development of lean tissue. This desirable effect is not obtainable with KIC although there is a slight tending for KIC to reduce the accumulation of body fat.

With reference to ruminants, HMB has the advantage of not being subject to appreciable rumen destruction. Consequently, HMB can be administered orally in defined amounts to ruminants, such as an additive to feeds for cattle or sheep, to obtain the growth metabolism effects and especially to increase the amount of lean tissues produced.

Advantageous growth metabolic effects can also be obtained by the feeding of HMB to non-ruminants, including poultry being raised for meat production, and chickens being fed for egg production. The use of HMB to increase the amount of lean meat less fat meat is also of importance with respect to pigs and chickens.

The desired growth effects described above can be obtained by the oral administration of calcium-HMB (Ca-HMB) or equivalent molar amount of other water-soluble non-toxic HMB salt. Amounts as low as 15 to 35 milligrams (mg) per kilogram (kg) of body weight per 24 hours can be used.

DETAILED DESCRIPTION

The compound used for practicing the present invention is beta -hydroxy- beta -methyl butyric acid or an edible butyrate salt thereof. The free acid compound is also called beta -hydroxy-isovaleric acid. It has the following structure:
[See Original Patent for Chemical Structure Diagram]

This compound in both free acid and salt form is referred to herein generically as "HMB". The acid form is designated HMB acid, and the specific salt form, such as the calcium or sodium salts, as Ca-HMB or Na-HMB. HMB has no isomers and accordingly does not exist in L or D forms. For the purpose of the present invention, it is preferred to employ HMB in the form of an edible salt rather than as the free acid. Preferably the salt form is water-soluble or becomes water-soluble in the stomach or intestines of the domestic animal. A preferred salt is the calcium salt (Ca-HMB). Sodium (Na-HMB) can also be used but Na-HMB is more hygroscopic than Ca-HMB. Other non-toxic salts can be used such as other alkali metal or alkaline earth metal salts. For mixing with feed ingredients, it is preferred that the salt form be dry, non-sticky, and finely-divided for blending with the feed materials. Ca-HMB is particularly desirable for these reasons.

HMB is not known to be commercially available. However, procedures are known for synthesizing this compound from commercially available starting materials. For example, HMB can be synthesized by oxidation of diacetone alcohol (4-hydroxy-4-methyl-2-pentanone). One suitable procedure is described by Coffman, et al., J. Am. Chem.Soc., 80:2882-2887, at 2885 (1958). As there described, beta -hydroxy-isovaleric acid (HMB) is synthesized by an alkaline sodium hypochlorite oxidation of diacetone alcohol. The product is recovered in free acid form, which can be converted to the desired salt. For example, HMB can be prepared as its calcium salt (Ca-HMB) by a similar procedure to that of Coffman, et al. in which the HMB acid obtained is neutralized with calcium hydroxide, and recovered by crystallization from an aqueous ethanol solution. For example, a 95% ethanol solution can be used with the Ca-HMB at about a 10% concentration. Such a procedure is illustrated in more detail in the following examples.

To assure administration at a desired level, it is preferred to mix the dry HMB salt with the dry feed ingredients to a predetermined concentration. The HMB

salt can be incorporated by dry blending using standard mixing equipment. The HMB should be substantially uniformly distributed throughout the feed. After mixing, if desired, the feed material may be further processed, such as by conversion to pellets.

Most feed compositions for domestic animals, such as for the raising of ruminants, swine, or poultry for meat production, are composed of mixtures of feed ingredients. These feed compositions contain protein-providing ingredients as principal components. These feed ingredients usually provide at least 10% protein by weight on a total dry matter basis, and may contain as much as 24% or more by weight. Such mixed feed compositions may comprise complete feeds or feed concentrates.

When HMB is combined with the feed material as a uniform mixture, and the feed composition provides the major food source for the diet, the amount of HMB may be specified in relation to the feed composition. For example, the admixed total ration feed compositions may contain from 0.001 to 0.5 wt % HMB (Ca-HMB and dry feed basis). On the same basis, a presently preferred range is from about 0.01 to 0.1 wt. % HMB. Such complete feed compositions will usually contain at least 10% protein and may contain up to 24% protein ($N \times 6.25$). For example, beneficial growth effects such as enhanced lean tissue development can be obtained in preferred embodiments by incorporating from about 0.02 to 0.04 wt. % (dry basis) of Ca-HMB or molar equivalent amount of another edible non-toxic water-soluble salt of HMB.

The foregoing feed composition levels of HMB relate primarily to feeds which are formulated to comprise substantially the total ration of the animal. Where feed supplements or feed concentrates are employed as the vehicle for administering the HMB, greater concentrations may be required. The concentrations can be similarly related to either the total diet of the animal, or to the body weight of the animals being fed.

It is believed that some beneficial effects can be obtained with as little as 0.05 to 0.2 milligrams (mg) of HMB (Ca-HMB basis) per kilogram (kg) body weight per 24 hours. It will usually be desirable, however, to administer at least 0.5 mg/kg body weight/24 hrs (Ca-HMB basis). It will not usually be necessary to administer more than 100 mg HMB (Ca-HMB basis) per kilogram of body weight per 24 hours, but higher amounts can be given up optimum range for most domestic animals is believed to be from about 15 to 35 mg/kg body weight/24 hrs (Ca-HMB basis).

As previously indicated, it is preferred to combine HMB with a complete feed ration, feed concentrate, or other dry feed material being given to the cattle, sheep, swine, chickens, or turkeys. In certain cases, however, HMB could be administered by dissolving it in drinking water for the animals. However, control of the amount administered in water can be expected to be more difficult. For more precise control, HMB could be orally administered in the form of pellets. For example, such pellets could be spread as a top dressing on the daily (24 hr) feed ration for each animal. Other methods of administration could be used.

As far as is known, HMB is not subject to significant rumen destruction. Following oral administration, the HMB salt appears to pass intact through the rumen into the intestines of the ruminant where it is absorbed. For the purpose of the present invention, it is believed that HMB should be absorbed into and distributed in the circulatory system.

In improving growth metabolism of domestic animals, several different effects

can be selected as a primary purpose of the HMB administration. For example, with respect to animals being raised for meat production, including beef cattle, such as steers and heifers, as well as lambs, and chicks, a principal objective can be to increase the development of lean tissue while minimizing the accumulation of body fat. Also a valuable effect for meat producing animals is to also improve feed efficiency, thereby producing more pounds of meat per pounds of feed. For dairy cattle or other lactating mammals, the feeding or oral administration of HMB in accordance with this invention can be used as a means of increasing milk production, or the feed efficiency for milk production. This can be evaluated in terms of the total amount of milk produced and/or the relation to the amount of milk produced per pound of feed. With respect to mature animals, such as sheep being fed for wool production or laying chickens being fed for egg production, an objective can be to increase the amounts of wool or eggs produced. This can be evaluated in relation to the total amount of wool or eggs produced, or the amount of wool or eggs produced per pound of feed. With reference to all of the foregoing uses or feeding objectives, it is believed that the HMB will also have a beneficial effect on the immune systems of the animals. In particular, HMB will activate the blastogenesis function of the immune systems of the domestic animals. This effect can be observed with respect to T lymphocyte function.

DETDESC:

The method of this invention and the feed compositions for use therein can be further illustrated by the following experimental examples.

EXAMPLE 1

Preparation of Ca-HMB

Reaction is contained in a 5000 ml round bottom flask fitted with condenser. Constant mixing is achieved by a magnetic stirrer.

Add in sequence:

1 gallon bleach (5.25% Sodium Hypochlorite)

45g NaOH powder

Mix well.

150 ml 1,4 Dioxane

93ml 4-Hydroxy-4-Methyl-2-Pentanone.

Reflux for 40 minutes.

Transfer solution to washtub and cool for 30 minutes under a hood.

Adjust pH to 5 using concentrated H₂SO₄. (HMB is stabilized.)

Transfer solution to cooling pans under a vent hood, so that air is drawn in over the solution for faster evaporation. Use the steam table if possible.

Let solution evaporate overnight. Sodium sulfate salts will precipitate out forming a slurry.

Transfer slurry to washtub, and adjust pH to 1 using concentrated H₂SO₄.

Salts and solution may be extracted separately depending on the volume available after evaporation.

Transfer solution with/without salts to 10 liter bottle, and wash 4 x with

approximately 2 liters of Ethyl Acetate. (Acetate layer contains HMB.)

Save ethyl acetate layer. Discard final acid layer.

Roto Vap Acetate layer at 50o C.

Salts may precipitate out.

Solubilize in Ultra-pure H2O.

Add an equal volume of Ethyl Acetate and re-extract. Save ethyl acetate layer.

Roto Vap Acetate layer at 50o C.

Increase Temperature to 70o C. and continue Roto Vap. Remaining solution contains HMB and is ready for crystallization.

The dried HMB acid is neutralized with calcium hydroxide powder. The powder is added to the stirring HMB acid until a basic pH is reached. The pH is assessed with pH paper. The HMB solidifies at this point and is subsequently dissolved in hot 95% ethanol at a volume of approximately equal to 10 times the original acid volume. Material that does not dissolve is removed from the liquid by centrifugation or filtration. The ethanol-HMB solution is then placed at -20°F until the mixture crystallizes. Usually this takes overnight but can take 2-3 days. The HMB crystals are then filtered under vacuum through paper towels and liquid squeezed out of the cake-like crystals. The HMB crystals are then redissolved in hot ethanol and the process repeated. In most cases 3 recrystallizations are sufficient to fully remove any yellow color from the crystals. Further purification can be achieved by further crystallization. After the final crystallization the HMB is placed in a pan and freeze-dried overnight to obtain an anhydrous calcium HMB powder ready for feed additive use.

EXAMPLE 2

Stability of HMB in the Rumen

Rumen fluid was collected from a fistulated steer. After filtration and dilution (1:4) with an artificial saliva, 25ml of the solution was added to 50ml plastic tubes. Each tube was fitted with a one-way valve to allow gases to escape while not allowing air into the tube. Each tube was then gassed with CO2 and incubated at 39o C. After 30 min a solution of KIC or HMB was added to the tube in concentrations to simulate what would be present in the rumen of an animal consuming 0.05% of the diet as KIC or HMB. It was estimated that a 50 uM concentration would be attained in this case. At timed intervals 50 ul samples of this rumen fluid were taken and analyzed for KIC and HMB. The results are shown in Table A.

TABLE A

Time after Addition (min)	* KIC	HMB
0	30*	60
15	15	76
30	4	81
60	2	71
240	2	74
480	1	74

n*Initial concentration of KIC could only be estimated. The initial concentrations should have been - 50 uM but because of the rapid degradation of

KIC in the rumen, KIC was already being degraded before the 0 time collection could be cooled and processed. -

The foregoing shows that KIC is rapidly destroyed by the rumen bacteria while HMB is quite stable in a rumen environment.

EXAMPLE 3

Feeding of HMB to Lambs-Trial 1 Materials and Methods

Animals and Experimental Design. Three sets of twin lambs were housed individually in fiberglass pens. Animals were randomly assigned to control or HMB diets, using Ca-HMB as the feed additive at a level of 0.5% of the basal diet.

TABLE B
Composition of Basal Diet Fed to All Sheep.

Ingredient	Kg Dry Matter Experiment 1
Ground corn	84.4
Expeller soybean meal	5.1
Molasses	6.5
Trace mineral & vitamin premix	0.66
Salt	0.66
Limestone	2.1
Urea	0.66

The results are summarized below in Table C.

TABLE C

	Renal Fat % (% of animal live weight)	Back Fat (in) (fat thickness over 12th rib)	Longissimus area (sq. in.)
Controls:	2.42	.21	2.1
HMB:	1.90	.12	2.3
% change due to HMB	- 22%*	- 43%*	+ 10%

	Average daily gain (kg/day)	Feed/Gain
Controls:	.287	3.89
HMB:	.296	3.60
% change due to HMB	+ 3%	- 7%

n*P < 0.5 -

As shown by the foregoing data, in this limited study HMB appeared to stimulate growth of lean tissues at the expense of fat. Both measures of fat decreased dramatically while the muscle weight increased substantially from controls.

EXAMPLE 4

Feeding of HMB to Lambs-Trial 2

Animals and Experimental Design. Twenty-two lambs were housed individually in fiberglass pens (1.15 m²) and randomly assigned to control or HMB diets. The control had nothing added to the diet while the HMB animals had 1 g of CaHMB supplemented to their diets each day. At the beginning of the experiment animals were weighed and ultrasound measurement made of the longissimus area and the amount of fat over the longissimus muscle. These measurements were made over the 12th rib area. Feed consumption was monitored. At the end of the experiment animals were weighed, feed consumption measured and the longissimus area and fat thickness measured again with the ultrasound apparatus. The gain in longissimus area and backfat were calculated from the initial value. This noninvasive method of estimating muscle and fat deposition has been shown to be a reliable method of assessing these values without slaughtering the animal.

TABLE D
Composition of Basal Diet Fed to All Sheep

Ingredient	Kg Dry Matter	
	Without HMB	With HMB
Ground corn	48.2	48.2
Dehydrated alfalfa	20.0	20.0
Expeller soybean meal	25.0	25.0
Molasses	5.0	5.0
Trace mineral & vitamin premix	0.2	0.2
Salt	0.5	0.5
Limestone	0.93	0.88
Ammonium chloride	0.30	0.30
Ca-HMB	0	0.050

Results:

TABLE E
The Effects of Feeding HMB to Young Lambs
from 60 lbs. to 100 lbs.

	Control	HMB	% Change
Total gain (lbs)	38.5	40.5	+ 6%
Feed/gain	4.41	4.28	- 3%
Loin area gain (cm ²)	2.08	3.34	60%**
Back fat gain (cm)	.27	.19	- 30%**

n**P < .02 level -

The foregoing data indicates that HMB supplementation increases preformance and increases lean vs. fat gain. These results are therefore corroborative of those of the first trial.

EXAMPLE 5

Feeding HMB to Poultry

To compare KIC and HMB as growth promotants, young male leghorn chicks were allotted to 12 battery (wire mess floors) pens consisting of 10 birds each. Treatments of control, HMB (0.04% of the diet) and KIC (0.04% of the diet) were assigned to the pens. Feed consumption was measured during the 5 week

experiment. During week 2 of the experiment chicks were injected with 50 ul of a 20% suspension of pig red blood cells intraperitoneally.

Chicks were fed a diet adequate in all nutrients for leghorns of this age. HMB was a supplement to this diet at 0.04% of the diet (w/w). The following diet was used as shown in Table F.

TABLE F

Ingredients (KG Each)	Control Diet	HMB Diet
Ground corn	59.3	59.3
Soybean meal	19.7	19.7
Wheat Middlings	13.4	13.4
Fish Meal	3.0	3.0
Calcium Carbonate	0.71	0.66
Dicalcium Phosphate	0.38	0.38
Meat and bone meal	3.0	3.0
Premix of vitamins & minerals	0.6	0.6
HMB	0.0	0.05

At the end of the 5 week experiment chicks were decapitated and serum collected for pig red blood cells antibody titer determination. Titers to red blood cells were evaluated by a microtiter agglutination test (Kuhlman, et al.). Also, the Pectoralis major muscle (the major breast muscle) was dissected and weighed as a measure of muscle growth. The data is summarized in Tables G1 and G2.

TABLE G1
Growth and Feed Efficiency of Male Leghorns
Fed KIC and HMB (5 week data)

	Control	KIC	HMB
Gain (g)	222	229	229
Feed/gain	2.30	2.24	2.17
Breast muscle (%)	2.84	2.94	3.00
Red blood cell titer (dilution)	1.57	2.65	3.25

TABLE G2

	KIC Change from Control	HMB Change from Control
Gain (g)	3.3%	3.3%
Feed/gain	- 2.5%	- 5.8%**
Breast muscle (%)	3.5%	5.5%**
Red blood cell titer (dilution)	68%	107%**

n**Significant at the $p < .02$ level -

The foregoing data shows that HMB increased performance of growing chicks by increasing the rate of growth and by making more efficient use of feed for growth. Additionally, it appears that muscle growth is stimulated relative to

other tissues as evidenced by increased breast muscle size. Compared to KIC, HMB was superior in all parameters measured.

Feeding Periods

To obtain the advantages of the method and feed compositions of this invention for selectively increasing lean tissue formation while minimizing fat deposition in the raising of meat producing animals, it will be preferred to feed the animals the HMB on a daily basis while the animals are increasing in weight, and to continue the feeding for at least 10 days and up to 180 days, depending on the animals being fed.

With beef cattle, for example, HMB is preferably fed for periods of at least 30 up to 180 days; with lambs, for periods of at least 15 up to 100 days; for chickens, for periods of at least 10 up to 50 days, and for turkeys, for periods of at least 10 up to 100 days. The periods referred to are when animals being raised are growing to market weight sizes. The maximum advantage of HMB administration should be obtained when fat being deposited and time deposition have decreased, which usually occurs in the later portion of feeding before marketing. Incorporation of HMB in finishing rations is therefore especially desirable.

CLAIMS: I claim:

[*1] 1. The method of raising meat producing domestic animals to increase lean tissue development as shown by muscle growth, said animals being selected from the group consisting of beef cattle, lambs, and poultry, comprising orally administering to said animals an effective amount of beta -hydroxy- beta -methylbutyric acid or an edible salt thereof (HMB) to promote lean tissue development as shown by muscle growth, said effective amount being within the range from 0.05 to 500 milligrams (mg) HMB (Ca-HMB basis) per kilogram (kg) of body weight per 24 hours, and said administration being continued for a sufficient length of time to obtain a substantial increase in lean tissue.

[*2] 2. The method of claim 1 in which the amount of said HMB on the same basis is within the range from 0.5 to 100 mg.

[*3] 3. The method of claim 1 or claim 2 in which said HMB is administered by admixing with feed for said animals, and the admixed feed is fed to the animals on a regular repeated basis for an extended number of days while the animals are increasing in weight.

[*4] 4. The method of feeding beef cattle to increase lean tissue development as shown by muscle growth, comprising feeding to said beef cattle in admixture with a protein-containing feed an effective amount of an edible salt of beta -hydroxy- beta -methylbutyric acid (HMB) to promote lean tissue development of said beef cattle as shown by muscle growth, said effective amount being within the range from 0.05 to 500 milligrams (mg) HMB (Ca-HMB basis) per kilogram (kg) of body weight per 24 hours, and said feeding being continued on a daily basis for at least 30 days while said beef cattle are gaining weight.

[*5] 5. The method of claim 4 in which the amount of said HMB salt is Ca-HMB and on said basis is fed within the range from 0.5 to 100 mg.

[*6] 6. The method of feeding lambs to increase lean tissue development as evidenced by muscle growth, comprising feeding to said lambs in admixture with a protein-containing feed an effective amount of an edible salt of beta -hydroxy- beta -methylbutyric acid (HMB) to promote lean tissue development as shown by muscle growth, said effective amount being within the range from 0.05 to 500

milligrams (mg) HMB (Ca-HMB basis) per kilogram (kg) of body weight per 24 hours, and said feeding being continued on a daily basis for at least 15 days while said lambs are gaining weight.

[*7] 7. The method of claim 6 in which said HMB is in the form of its calcium salt (Ca-HMB) and said effective amount of Ca-HMB is within the range from 0.5 to 100 mg.

[*8] 8. The method of raising poultry for meat production to increase the development of lean tissue as shown by muscle growth, said poultry being selected from the group consisting of chickens and turkeys, comprising feeding said poultry an effective amount of an edible salt of beta -hydroxy- beta -methylbutyric acid (HMB) to promote lean tissue development as shown by muscle growth, said effective amount being within the range from 0.05 to 500 milligrams (mg) HMB (Ca-HMB basis) per kilogram (kg) of body weight per 24 hours, and said feeding being continued on a daily basis for at least 10 days while said poultry are increasing in weight.

[*9] 9. The method of claim 8 in which said HMB is in the form of its calcium salt (Ca-HMB) and said HMB is fed in an amount from 0.5 to 100 mg on said basis.

[*10] 10. The method of claims 8 or 9 in which said poultry are chickens.

[*11] 11. The method of claims 8 or 9 in which said poultry are turkeys.
